Message

From: Lazarus, Rebecca [lazarus.rebecca@epa.gov]
Sent: 11/19/2021 3:10:49 PM
To: Sankula, Sujatha [Sankula.Sujatha@epa.gov]; Jones, Kristin [jones.kristin@epa.gov]; Steeger, Thomas [Steeger.Thomas@epa.gov]; Farruggia, Frank [Farruggia.Frank@epa.gov]; Thompson, Pamela [Thompson.Pamela@epa.gov]; Stebbins, Katherine [Stebbins.Katherine@epa.gov]; Hartless, Christine [Hartless.Christine@epa.gov]; Milone, Joseph [Milone.Joe@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]
Subject: SETAC Pollinator Content

Good morning pollinator team,

I wanted to share some of the relevant honey bee content I came across at SETAC this past week. The last two are butterfly ones.

Best regards,
Rebecca

03.04.06 - A Semi-Field Colony Feeding Study Using the Common Eastern Bumble Bee (Bombus impatiens)

- *Recording available*

Daniel Schmehl[1], Leif Richardson[2], Ana Cabrera[1] and Pamela Jensen[3], (1)Bayer CropScience, (2)Stone Environmental, Inc., (3)Bayer CropScience LP

Ecological risk assessment is a key component of the regulatory process required for registration of crop protection products in the US and elsewhere. The western honey bee (Apis mellifera) is the model test organism for pesticide risk assessments to pollinating bees, yet there is uncertainty around whether it is protective for other bees in all circumstances. Accordingly, efforts are underway in Europe to adapt honey bee test methodologies for two types of native bees, bumble bees and mason bees. To address the need to develop colony level methodology for bumble bees, in 2020 we conducted a semi-field colony feeding study with the common eastern bumble bee (Bombus impatiens) in agricultural landscapes in central Vermont. Adapting recently developed semi-field study protocols for honey bee colony feeding studies, we exposed commercially available bumble bee colonies to four concentrations of dimethoate insecticide (0.05, 0.19, 0.75, and 3.00 ppm) delivered via supplemental sugar solution, and compared these colonies to controls. We established 10 sites in three clusters around the margin of hayfields where pesticide usage was low and row crop agriculture accounted for no more than 20% of land use in the range of bumble bee foragers. Each treatment group was represented by one colony at each apiary. Hives were free to forage naturally, and were provisioned and inspected weekly over the course of the summer. We collected data on a range of individual- and colony-level endpoints relevant to bumble bee life history, including production of female reproductive (gyne) offspring, colony mass, foraging activity, and consumption of provisioned sugar solution. We found that dimethoate consumption exerted a concentration-dependent effect on these response variables, with the level of No Observed Adverse Effects Concentration (NOAEC) for most determined as 0.10 ppm dimethoate. Our work is novel in that it is the first fully-replicated semi-field colony feeding study to be conducted using B. impatiens, a key component of future risk assessment research for non-Apis bees in North America. We anticipate that our methods and results can guide future efforts to develop a standard test paradigm to assess risk of crop protection products to bumble bees.

01.20.17 - Systemic Pesticides in Japanese Orchard Bee (Osmia cornifrons) Pollen Stores Affect Larval Development and Increase Pupal Mortality - ***E-Poster Available***

Ngoc Phan[1], Ed Rajotte[1], Neelendra Joshi[2] and David Biddinger[3], (1)Penn State University, (2)University of Arkansas, (3)Pennsylvania State University

Solitary bees, while providing pollination services, are often exposed to various pesticides applied for pest control on farmland. There is increasing evidence that sublethal toxicity of agricultural pesticides affects solitary bees differently than social bees (e.g., honey bees and bumble bees). Solitary bee studies are challenging because of the difficulties in obtaining large numbers of eggs or young larvae and due to their univoltine life cycle. Here we show the toxic and sublethal developmental effects of four widely used plant systemic pesticides on the Japanese orchard bee (*Osmia cornifrons*). Pollen stores of this solitary species were collected and treated with different concentrations (1/10X, 1X, and 10X) of three insecticides (acetamiprid, flonicamid, and sulfoxaflor) and a fungicide (dodine) based on previously measured field-realistic concentrations in apple orchard pollen. Eggs were transplanted to the treated pollen in rearing chambers and hatching larvae were allowed to feed on the pollen stores. The effects of chronic ingestion of contaminated pollen were measured until adult eclosion. This year-long study revealed that chronic exposure to all tested pesticides caused delayed larval development, lowered larval and adult body weights. Additionally, exposure to the systemic fungicide resulted in abnormal larval defecation and increased mortality at the pupal stage, indicating potential threats to bees from fungicide exposure. These findings highlighted potential new threats to solitary bees from systemic insecticides and fungicides and will be helpful in understanding and mitigating these effects.

03.08.06 - Evaluation of In Vitro Toxicity of Organophosphate Insecticide, Dimethoate on Honeybee Larvae (Apis mellifera L.) by Acute and Chronic Exposure

- *Recording available*

Jigarkumar Rana, Pritee Singh, Alka Rai, Minal Kamle and Padmaja Prabhu, Jai Research Foundation, India

The Honeybee, *Apis mellifera* L. is present worldwide. It plays a very important role as pollinator for agricultural crops and for wild plants. Loss of honeybee colonies and disorders are observed in many countries. The current situation threatens the continuity of pollinators role and the balance in nature. Multiple factors have been attributed to this decline, which includes parasites, pathogens, poor nutrition, queen failure, habitat loss and migratory stress and extensive use of pesticides on crops. Among these factors, the potential impact of pesticides, particularly those applied in agricultural settings, are of particular interest to us. In the current research, organophosphate insecticide, dimethoate (DMT) has been used. The aim of the current research is to evaluate the toxicity on honeybee larvae (*Apis mellifera* L.) with acute and chronic exposure of DMT. Protein and energy are required for honeybee growth and development. This requirement is fulfilled with pollen and nectar stored in the hive which is carried by worker honeybees. During foraging, honeybee carries pesticides in the hive which exposed to nurse bees to feed the larvae. In the recent work, we exposed DMT to honeybee larvae (*in vitro*) in single (acute) and repeated exposure (chronic). Honeybee larvae were exposed with DMT at the dose levels of 0.0 (control), 0.13, 0.25, 0.50, 1.00, 2.00, and 4.00 µg/larvae, in single exposure and at the dose levels of 0.0 (control), 0.4, 1.1, 3.3, 10.0, and 30.0 mg a.i./kg diet, in repeated exposure. Larvae were observed for mortality, behavioural symptoms, morphological differences and adverse effects after emergence. A significant decrease in survival was observed between 0.13 and 4.00 µg/larvae in single exposure and between 1.1 and 30.0 mg a.i./kg diet. These results represent the assessment of the effects of DMT in honeybee larvae and should contribute to studies on honeybee colony decline. Overall, our findings are valuable for assessing the acute and chronic toxicity of developing honeybee.

05.13.11 - Compilation and Statistical Analysis of Pollen and Nectar Pesticide Residue Levels: Applications to Tier 1 and Refined RUDs for Pesticide Risk Assessment ***- E-Poster Available***

Larry Brewer, Terrestrial Ecotoxicology, Compliance Services International, Lakewood, WA, Timothy Fredricks, Bayer CropScience, Silvia Hinarejos, Sumitomo Chemical Agro Europe, France, Max Feken, Syngenta, Timothy Joseph, Landis International, Inc., Afghanistan, Verissimo Sa, Dow AgroSciences and William Warren-Hicks, ECOSTAT

Honey bee dietary risk assessment of pesticides requires knowledge of the residue levels in nectar and pollen, either following foliar application to crops, trunk/stem injection, soil application or seed treatment. Current Tier 1 bee risk assessment in the United States relies on an exposure estimation and risk assessment model called BeeREX. This model uses a Residue Unit Dose (RUD) approach to estimate residues in nectar and pollen based on the upper-bound pesticide residue values from US Environmental Protection Agency's (US EPA) T-REX model (version 1.5) of residues measured on a variety of plant matrices assembled for the purpose of dietary risk assessment in birds and mammals. Specifically, the RUD for 'long grass' residues are used within BeeREX as a surrogate for residues in nectar and pollen. In comparison, European Union (EU) Tier 1 risk assessment uses a database of nectar and pollen residue data. The US EPA has recently received residue study data from several applicants that can be used to adequately describe the distribution of pesticide residues that occur in pollen and nectar relative to application rate, method of application, and crop. By combining the EU and US EPA variety of plant matrices, especially nectar and pollen databases a statistically refined estimation of RUD values can be calculated. The calculated nectar and pollen RUD values will then inform the BeeREX model with exposure data relevant to the bee risk assessment.

05.13.15 - Pesticide Presence, Land Use, and Honey Bee Hive Health in North-Central Oklahoma ***- E-Poster Available***

Carrie Klase, Jason Belden and Kristen Baum, Oklahoma State University

Honey bees (*Apis mellifera*) are pollinators of a variety of crop species. With declines in the number of commercial hives, understanding the factors that impact honey bee survival and health is crucial to maintaining agricultural production. Managed honey bee hives are frequently placed on or near crop fields to provide pollination services, potentially exposing them to pesticides. Exposure to pesticides can occur through two primary routes: external/physical contact (which can be assessed through surface residue of pesticides) and diet (which can be assessed through pollen collected by honey bees). Our objective is to determine whether the presence of pesticides influences honey bee health across three land use types (canola crop, winter wheat crop, and grassland). We are evaluating if pesticides occur in pollen from hive pollen traps and on the surfaces of surrounding wildflowers and crops and, if so, at what concentrations. We are also assessing whether hive health (measured as weight changes and *Varroa* mite parasite loads) is affected by pesticide presence and concentrations. We placed three hives at 24 study sites (12 sites in 2019, four near each land use type, and repeated at different site locations in 2020) in north-central Oklahoma. Hives remained at the sites from March to early October, and samples were collected three times during that time period (late April/early May during peak canola bloom, July during post-canola harvest, and late September). During each sampling period, we collected samples to assess pesticide surface residue of crops and wildflowers, pesticides in pollen from hive pollen traps, hive weights, and *Varroa* mite loads in each hive. We expect that as quantities and concentrations of pesticides increase, the *Varroa* mite loads will increase and the honey bee hive weights will decrease. We also expect that honey bee hives at grassland sites will consistently have greater weight increases and fewer parasites compared to honey bee hives at winter wheat sites and canola sites, although hives at canola sites will initially gain the most weight due to floral resource availability during peak canola bloom.

05.20.03 - BEEHAVEecotox - a Mechanistic Effect Model for Honeybees - *E-Poster Available*

Thomas Preuss[1], Annika Agatz[2], Benoît Goussen[3], **Vanessa Roeben**[4], Jack Rumkee[5], Liubov Zakharova[2] and Pernille Thorbek[6], (1)Bayer Ag, Germany, (2)IBACON GmbH, Germany, (3)ibacon GmbH, Rossdorf, Germany, (4)Bayer AG, Germany, (5)Syngenta, United Kingdom, (6)BASF SE, Germany

Bees are important pollinators and thus an essential part of the environmental risk assessment of pesticides in the EU and in the US. Here, we introduce BEEHAVEecotox; an ecotoxicological model that mechanistically links exposure of bees in the field with the hazard profile for individual honeybees, leading to emerging colony effect. It is an addition to the widely used and extensively tested BEEHAVE colony model. The mechanistic link allows the translation of results from standard laboratory studies to relevant processes and parameters for simulating bee colony dynamics. The BEEHAVEecotox model includes 4 submodules: an external exposure module, in-hive fate module, water foraging module, and an effect module. The external exposure module incorporates the concentration of PPPs in the bee-relevant matrices such as nectar, pollen, and water. When foragers forage on these matrices, they receive an oral dose of the PPP, and they can be exposed via contact on the day of application. The water foraging module incorporates the need for water for cooling of the hive and dilution of stored honey, including potential exposure to PPPs. The in-hive fate module simulates the entry and mixing of the PPP into the hive, through nectar, pollen, or water. The effect module covers the mortality due to exposure for the different cohorts. It uses the slopes and LD50 values of standard acute contact, oral, chronic oral, and larvae studies as inputs. The BEEHAVEecotox model was validated against two semi-field studies with a tunnel setup with two PPPs with different modes of action (dimethoate and fenoxycarb). The validation showed that the BEEHAVEecotox model captured the initial effects on colony strength and the subsequent colony dynamics well for both substances. The model predicted the relative magnitude of effect at colony level directly after application, as well as the long-term reduction in colony strength in the post-exposure phase after the tunnel and the lack of recovery of the colony. The BEEHAVEecotox model is a suitable tool to predict the effects of PPPs on bees. It is the first model to mechanistically predict PPP exposure to foragers and within the hive from several different routes of exposure. For the regulatory risk assessment the model can potentially be used to extrapolate from laboratory to semi-field and field studies. Furthermore, it offers the possibility to study the effects in different crops and regions and to test different mitigation strategies.

03.08.02 - Assessing Monarch Caterpillar (Danaus plexippus L.) Survival From Exposure to Fungicide-Insecticide Combinations

- ***Recording available***

Matthew Greiner[1], Annie Krueger[1], Autumn Smart[2], Tom Weissling[2], Troy Anderson[3] and Ana Velez[3], (1)University of Nebraska Lincoln, (2)University of Nebraska-Lincoln, (3)University of Nebraska – Lincoln

The intensively cultivated Midwestern U.S. is a crucial breeding ground for the monarch butterfly (*Danaus plexippus* L.). Monarch butterfly larval host plants, *Asclepias* spp., frequently occur near agricultural fields and pesticide residues are often present on these milkweeds. Therefore, monarch caterpillars present on *Asclepias* spp. close to agricultural fields are likely exposed to multiple pesticides. Previous studies with the honey bee, *Apis mellifera*, indicated synergistic interactions between demethylation-inhibiting (DMI) fungicides and pyrethroid, anthranilic diamide, and neonicotinoid insecticides, leading to higher mortality. The increased mortality caused by DMI fungicides and insecticide combinations is hypothesized to occur via the inhibition of cytochrome P450 detoxification enzymes by DMI fungicides. Interactions between DMI fungicides and insecticides in monarch caterpillars have not yet been studied. This research focuses on evaluating the interactions between a prominently used DMI fungicide, propiconazole, and the insecticides bifenthrin, chlorpyrifos, and thiamethoxam in monarch caterpillars. This study evaluated 3rd-instar monarchs orally exposed to combinations of propiconazole and each insecticide via incorporation into an artificial diet. Mortality, behavior, and morphology were recorded daily for 96 hours. After the 96-hour exposure, the caterpillars were observed until pupation and adult emergence to record lethal and sublethal effects. Pesticide exposures were based on worst-case scenarios for spray drift in agricultural ditches via modeling pesticide deposition at 0, 5, and 10 meters from application fields using the high spray label rates of formulated products with the AgDRIFT program. The results from this research demonstrate plausible impacts of exposure to fungicide-insecticide combinations on monarch caterpillars developing close to agricultural fields.

03.04.05 - Estimating Screening-Level Risks of Insecticide Exposure to Lepidopteran Species of Conservation Concern in Agroecosystems

- ***Recording available***

Maura Hall, Niranjana Krishnan, Joel Coats and Steven Bradbury, Iowa State University

The U.S. Fish and Wildlife Service defines 'at-risk' species as those that have either been petitioned for listing, proposed for listing, or assigned a candidate species status under the Endangered Species Act. In the lower 48 United States, there are currently 24 butterflies listed as endangered. Of these species, the Dakota skipper (*Hesperia dacotae*), Karner blue (*Lycaeides melissa samuelis*) Mitchell's satyr (*Neonympha mitchellii mitchellii*), and Poweshiek skipperling (*Oarisma poweshiek*) are found in the north central states, in addition to the monarch butterfly (*Danaus plexippus*), which was listed as a candidate species in 2020. Loss of habitat and exposure to pesticides, particularly insecticides, are considered threats to population recovery for all five of these species. Given the range of these at-risk species, re-establishment of habitat in agricultural landscapes is typically identified as a primary conservation practice to support recovery. To evaluate conservation risks and benefits associated with habitat placed in close proximity to crop fields, estimates of exposure and toxicity of insecticides are needed for these lepidopteran species. Here we present preliminary screening-level risk analyses for lepidopteran species of conservation concern, based on an evaluation and integration of environmental monitoring and toxicity studies reported in the peer-reviewed literature. We interpreted the utility of existing insecticide residue data to estimate species-specific larval host plant exposure. Based on available lepidopteran toxicity data, we developed Species Sensitivity Distribution (SSD) models for topical exposures to pyrethroid and organophosphate insecticides; inadequate data were available for other classes of insecticides and dietary exposures. Using the generated SSD models with the available exposure data, we explored potential insecticide risks associated with establishing non-target lepidopteran habitat in agricultural landscapes. We also discuss the kinds of toxicity data needed to generate more models and reduce uncertainties in model predictions, and identify needs for future monitoring studies to address exposure data gaps.